OPP OFFICIAL RECORD
HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS
EPA SERIES 361

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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF PREVENTION, PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

Date: January 22, 2009

SUBJECT: Clothianidin: Developmental Immunotoxicity Study

PC Code: 044309
Decision No.: 400521
Petition No.: NA
Risk Assessment Type: NA
TXR No.: 0054981
MRID No.: 47526501

DP Barcode: D356839
Registration No.: NA
Regulatory Action: NA
Case No.: NA
CAS No.: 210880-92-5
40 CFR: NA

Ver.Apr.08

FROM: **Yung G. Yang, Ph.D.** Yung G. Yang.
Toxicology and Epidemiology Branch
Health Effects Division (7509P)

THROUGH: **Mary Manibusan, Chief**
Toxicology and Epidemiology Branch
Health Effects Division (7509P)

TO: **William T. Drew, Risk Assessor**
Risk Assessment Branch II
Health Effects Division (7509P)
And
Kable Davis, Risk Manager Reviewer
Insecticide/Rodenticide Branch, RM 01
Registration Division (7505P)

I. CONCLUSIONS

Under conditions of this study, there were no immunologically adverse effects on humoral or cell-mediated immunity in male and female rats that exposed to clothianidin during the prenatal, postnatal and post-weaning period. This study has fulfilled the data requirement for a developmental immunotoxicity study.

JAN 29 REC'D, 2009

II. ACTION REQUESTED

The Registration Division (RD) requested the Health Effects Division (HED) to review a developmental immunotoxicity study (MRID 47526501) submitted by the registrant to fulfill the condition of registration for clothianidin.

III. BACKGROUND

Previously, the HIARC reviewed the toxicity database of clothianidin and noted evidence of potential effects on the immune system in several studies (decreased absolute and relative thymus and spleen weights). Juvenile rats in the reproduction study appeared to be more susceptible to these effects. Therefore, the HIARC recommended that testing be conducted to assess immune system function in adults and in young animals following exposure during the period of organogenesis. A guideline adult immunotoxicity study had been conducted and showed no clothianidin-mediated immunotoxicity at doses lower than those resulting in generalized signs of toxicity (decreases in body weight); however, it cannot be concluded that a similar lack of effects will occur in offspring. Based on evidence of decreased absolute and adjusted organ weights of the thymus and spleen in multiple studies in the clothianidin database, and on evidence of increased quantitative susceptibility of juvenile rats to these effects (compared to adults) in the two-generation reproduction study, HED has recommended that a developmental immunotoxicity study (DIT) be conducted.

A developmental immunotoxicity study was conducted and submitted to fulfill the condition of registration for clothianidin. This study was sponsored by Sumitomo and monitored by Bayer CorpScience.

IV. RESULTS/DISCUSSION

The Toxicology and Epidemiology Branch (TEB) has reviewed the study and considered the study as acceptable/non-guideline. Under conditions of this study, there were no immunologically adverse effects on humoral or cell-mediated immunity in male and female rats that exposed to clothianidin during the prenatal, postnatal and post-weaning period. An executive summary is as follows.

EXECUTIVE SUMMARY: In a non-guideline developmental immunotoxicity study (MRID 47526501), 25 presumed pregnant Sprague-Dawley rats/dose group were dosed with Clothianidin (95.2% a.i.; batch no. 30037120) in acetone as dietary admixtures at doses of 0, 150, 500, or 2000 ppm. These doses corresponded, respectively, to doses of 0, 10, 35, or 121 mg/kg/day for gestation day (GD) 6-20 and 0, 22, 68, or 250 mg/kg/day for lactation day (LD) 0-13. After weaning (PND 22 to 36), F1-generation rats were offered the dietary formulations at the same concentrations as their parents and the corresponded doses were 0, 28/26, 98/93, or 404/404 mg/kg/day for males/females in Assay 1 and 0, 28/27, 89/93, or 338/398 mg/kg/day for males/females in Assay 2, respectively. Prior to PND 21, the F1-generation were potentially exposed through the maternal milk and inadvertently to the dam's dose formulation after approximately PND 13. Following weaning, one pup/sex/litter was selected to continue on study when possible. Two to three weanlings/sex/dose were evaluated for the following parameters:

mortality, body weight, body weight gain, food consumption, and gross pathology (sacrificed at approximately 7 weeks). The remaining weanlings were assigned to immunoassays.

Weanlings assigned to Assay 1 (antibody-forming-cell assay) were sensitized by intravenous injection of 0.5 ml of sheep erythrocytes (SRBCs) 4 days before sacrifice. Rats were sacrificed at 41±5 days of age and the thymus and spleen were excised and weighed. The primary IgM anti-SRBC antibody-forming-cell responses were determined. Weanlings assigned to Assay 2 (delayed-type hypersensitivity, DTH) were sensitized by subcutaneous injection of 0.2 ml *Candida albicans* formalin fixed cells in their right flank 8 days before being challenged with 100 µl injection of *Candida albicans* chitosan antigen into the foot pad. Swelling of the footpads, due to DTH response, was measured with a micrometer prior to challenge and 24 and 48 hours after the challenge. A challenge-only control group was used to correct for trauma associated with the challenge injection. Cycloposphamide (CPS) served as a positive control. CPS was administered at a dose of 50 mg/kg (10 mg/mL) in phosphate buffered saline by daily intraperitoneal injection on the last 4 days of exposure for Assay 1 and on the last 4 days before challenge for Assay 2.

For maternal toxicity, no treatment-related effects were observed on mortality or gross pathology in the dams. At 2000 ppm in the dams, an increased incidence of ptosis was observed in 6 animals for a total of 16 observations compared to 0 animals in the other groups. At 2000 ppm, the body weights were generally decreased during GD 9-20 by 3-6% and during LD 0-21 by 3-11%. Body weights gains were decreased during GD 6-9 by 71% and during GD 18-20 by 25%. These effects contributed to a decreased body weight gain of 21% for the overall (GD 6-20) treatment interval and a decreased body weight gain in the overall (GD 0-20) gestational period of 14%. During the lactation period a weight loss was noted during LD 0-3 (-8.0 g) compared to controls (4.6 g), and body weight gain remained decreased for LD 3-6 by 58%. Food consumption was decreased during the overall treatment, gestational, and lactation periods.

The maternal LOAEL is 2000 ppm, based on increased incidence of ptosis, and decreased body weights and body weight gain, and food consumption. The maternal NOAEL is 500 ppm.

For offspring toxicity, no treatment-related effects were observed on litter size and litter viability. No mortality or clinical signs of toxicity were observed in the offspring after weaning. At 2000 ppm, mean pup weights in the treated groups were decreased throughout lactation by 12-26%. After weaning, decreased body weight and body weight gains were generally observed until termination (↓7-29%), and bodyweight gain from PND 22 until termination was decreased ($p \leq 0.01$) by 24-25% in males and 15-17% in females. Food consumption was decreased for PND 22-29, 29-36, and 22-36 by 19-34%.

The offspring LOAEL is 2000 ppm, based on a decreased body weights, body weight gains, and food consumption. The offspring NOAEL is 500 ppm.

For immune function evaluation, there were statistically significant decreases in thymus and spleen weight in the F1 weanlings at 2000 ppm for males and females. These decreases might be related to significant decreases of body weights at the high dose and was considered secondary effect to general systemic toxicity and not direct immunological toxicity.

The antibody-forming cell assay (humoral immunity) in males showed decreases in total spleen cell number, spleen specific activity (AFC/10^6 spleen cells) and total spleen activity (AFC/spleen) at 2000 ppm while increased activities were observed at 150 and 500 ppm; there were no statistical significances with the treated groups compared to controls. In contrast, females showed statistically significant increases ($p \leq 0.05$) in spleen specific activity (AFC/10^6 spleen cells, ↑143%) and total spleen activity (AFC/Spleen, ↑99%) at 2000 ppm. However, individual animal data showed that the majority of animals in this group showed similar response compared with other treated and control groups except 3 females in the 2000 ppm that had higher specific activity and total spleen activity than the median response of this group that resulted in the statistically significant increased activities of this group compared to the controls. The variability of immune response was commonly observed in the outbred SD rats. Therefore, the statistical significance may not be biologically significant considering with distribution pattern of the responses. The positive control induced the expected response that produced significant decreases in total spleen cell number, specific activity and total spleen activity compared with the control.

For cell-mediated immunity, delayed-type hypersensitivity (DTH) assay did not show treatment-related effect at any treated dose at both 24 and 48 hours in this assay for males and females. The positive control showed the expected response of significantly suppressive responses at 24 hour and 48 hour post-challenge.

Under conditions of this study, there were no immunologically adverse effects on humoral or cell-mediated immunity in male and female rats that exposed to clothianidin during the prenatal, postnatal and post-weaning period.

This study is classified **acceptable/non-guideline** and provides information for use in evaluating the potential for immunotoxic effect in offspring rats after exposure to clothianidin during the prenatal, postnatal and post-weaning period.

DATA EVALUATION RECORD

CLOTHIANIDIN (TI 435)

Study Type: Non-guideline Developmental Immunotoxicity Study in Rats

Work Assignment No. 5-1-201 (MRID 47526501)

Prepared for
Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
2777 South Crystal Drive
Arlington, VA 22202

Prepared by
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Sciences Division
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Quality Assurance: Steven Brecher, Ph.D., D.A.B.T.	Signature: Date: 12/01/08

Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel

EPA Reviewer: Yung G. Yang, Ph.D. **Signature:** Yung G. Yang
Toxicology and Epidemiology Branch, Health Effects Division (7509P) **Date:** 1/21/2009
Work Assignment Manager: Myron Ottley, Ph.D. **Signature:** MOttley
Risk Assessment Branch III, Health Effects Division (7509P) **Date:** 1/21/09
Template version 02/06

DATA EVALUATION RECORD

STUDY TYPE: Developmental Immunotoxicity Study in Rats (diet)- Non-guideline

PC CODE: 044309 **DP BARCODE:** D356839
TXR #: 0054981 **SUBMISSION:** S835361

TEST MATERIAL (PURITY): Clothianidin (95.2% a.i.)

SYNONYMS: TI 435, [C(*E*)]-*N*-[(2-chloro-5-thiazolyl)methyl]-*N'*-methyl-*N''*-nitroguanidine

CITATION: Hobeman, A.M. (2008). Oral (diet) developmental immunotoxicity study of TI 435 (clothianidin) in Crl: CD(SD) rats. Charles River Laboratories, Preclinical Services, Horsham, PA. Laboratory Study no.: 5819-008, August 6, 2008. MRID 47526501. Unpublished.

SPONSOR: Sumitomo Chemical Company, Ltd., 27-1, Shinkawa 2-chome, Chuo-ku, Tokyo, Japan

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100 µl injection of *Candida albicans* chitosan antigen into the foot pad. Swelling of the footpads, due to DTH response, was measured with a micrometer prior to challenge and 24 and 48 hours after the challenge. A challenge-only control group was used to correct for trauma associated with the challenge injection. Cyclopposphamide (CPS) served as a positive control. CPS was administered at a dose of 50 mg/kg (10 mg/mL) in phosphate buffered saline by daily intraperitoneal injection on the last 4 days of exposure for Assay 1 and on the last 4 days before challenge for Assay 2.

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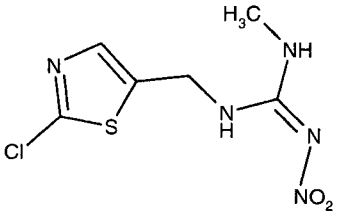
This study is classified **acceptable/non-guideline** and provides information for use in evaluating the potential for immunotoxic effect in offspring rats after exposure to clothianidin during the prenatal, postnatal and post-weaning period.

COMPLIANCE: Signed and dated Quality Assurance, Data Confidentiality, GLP Compliance, and Flagging statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Clothianidin

Description:	Not reported
Batch #:	30037120
Purity:	95.2% a.i.
Compound stability:	Stable for at least 21 days at room temperature and under refrigerated conditions
CAS #:	210880-92-5 (formerly 205510-53-8)
Structure:	

2. Vehicle: Diet with test compound in acetone

3. Positive control: Cyclophosphamide in phosphate buffered formalin (for the immunoassays)

4. Test animals

Species:	Rat	
Strain:	Crl:CD®(SD)	
Age/ weight at GD 0:	Approximately 71 days old; weighing 204-237 g	
Source:	Charles River Laboratories, Inc., Portage, MI	
Housing:	Female rats were individually housed in nesting boxes beginning no later than gestation day (GD) 20 until delivery and continuing until weaning (or until sacrifice at GD 25 for dams that did not deliver). Otherwise, rats were housed individually in stainless steel, wire bottomed cages	
Diet:	Certified Rodent LabDiet® 5002 (PMI Nutrition International, Inc., St. Louis, MO), *ad libitum*	
Water:	Tap water, purified by reverse osmosis then chlorine additive, *ad libitum*	
Environmental conditions:	**Temperature:**	64-79°F
	Humidity:	30-70%
	Air changes:	≥10/hour
	Photoperiod:	12-hour light/dark cycle
Acclimation period:	6 days	

B. PROCEDURES AND STUDY DESIGN

1. Purpose: The purpose of this study was to provide information for use in evaluating the potential for immunotoxic effects in offspring of rats after exposure to Clothianidin *in utero* and/or via maternal milk and diet during the lactation period and continuing through approximately 7 weeks of age.

2. In life dates: Start: Approximately November 4, 2007 End: January 4, 2008

3. Mating: Sexually mature, nulliparous females were mated overnight with males of the same strain and source at a ratio of 1 female: 1 male. Mating was verified by the presence of a vaginal copulatory plug or the presence of sperm in a vaginal smear. The day on which

evidence of mating was detected was designated gestation day (GD) 0. The cohabitation period consisted of a maximum of 5 days.

4. **Animal assignment, study design, and dose administration:** Presumed pregnant dams were assigned randomly, stratified by body weight, to the treatment groups shown in Table 1.

TABLE 1a. Animal assignment [a]				
Dose (ppm)	**0 (carrier)**	**150**	**500**	**2000**
No. P females	25	25	25	25
No. F1 rats/sex not selected for immunological examination	2	3	3	3
No. F1 rats/sex tested for antibody-forming cell response to sheep erythrocytes [b] (Assay 1)	20	20	20	20
No. F1 rats/sex tested for delayed-type hypersensitivity [c] (Assay 2)	20	20	20	20

a Data were obtained from pages 34-36 of the study report.
b Positive control (cyclophosphamide, 50 mg/kg; 10 mg/mL) in phosphate buffered saline was also tested, 10 rats/sex.
c Two additional control groups were tested. One group received carrier (acetone) and was challenged with *Candida albicans* chitosan antigen. Another group (data reported for 9 males and 10 females) received cyclophosphamide (50 mg/kg; 10 mg/mL) in acetone and was similarly challenged.

TABLE 1b. Consumed dosages (mg/kg/day) in dams [a]			
Dose (ppm)	**150**	**500**	**2000**
GD 6-20	10.4	35.0	120.6
LD 0-13	22.3	68.3	249.7

a Data were obtained from page 45 of the study report.

TABLE 1c. Consumed dosages (mg/kg/day) in males/females for the F1-generation rats used in the immunoassays [a]			
Dose (ppm)	**150**	**500**	**2000**
Assay 1 (PND 22-36)	27.5/26.4	97.9/92.9	403.7/404.4
Assay 2 (PND 22-36)	28.2/26.8	88.9/92.6	337.7/398.0

a Data were obtained from page 49 of the study report.

The formulations were presented to the P-generation rats beginning on GD 6 and continuing through GD 24 (rats not delivering) or lactation day (LD) 21, after which the adults were sacrificed. The following parameters were evaluated: mortality, clinical signs, body weight, body weight gain, food consumption, gross pathology, number of implantation sites, live and stillborn pups, litter size, and pup viability. On PND 3, a randomization program was used to select F1 generation pups to be culled, and litters were reduced to 10 pups each on PND 4.

The F1-generation rats were offered the formulations beginning on PND 21 and continuing until the scheduled termination of the animals. Prior to PND 21, the F1-generation were potentially exposed through the maternal milk and inadvertently to the dam's dose formulation after approximately PND 13. Following weaning, one pup/sex/litter was selected to continue on study when possible. Two to three weanlings/sex/dose were evaluated for the following parameters: mortality, body weight, body weight gain, food consumption, and gross pathology (sacrificed at approximately 7 weeks). The remaining weanlings were assigned to immunoassays.

In the immunoassays, cyclophosphamide (CPS) served as a positive control. CPS was administered daily at a dose of 50 mg/kg (10 mg/mL) in phosphate buffered saline by intraperitoneal injection on the last 4 days of exposure for Assay 1 and on the last 4 days before challenge for Assay 2.

Weanlings assigned to Assay 1 were administered a single intravenous injection of sheep erythrocytes (SRBCs) 4 days before sacrifice. The thymus and spleen weights, cells/spleen, antibody forming spleen cells (AFC) per 1 million spleen cells, and AFC/spleen were determined. Weanlings assigned to Assay 2 were administered *Candida albicans* formalin fixed cells in their right flank 8 days before being challenged with an injection of *Candida albicans* chitosan antigen into the foot pad. The foot pad was measured with a micrometer prior to challenge and 24 and 48 hours after the challenge. Unneeded pups were sacrificed on PND 3 or 4, while weanlings used in the immunoassays were sacrificed at approximately 6-7 weeks of age.

5. **Dose-selection rationale:** Doses were selected based upon the results of a 2-generation reproductive toxicity study (MRID 45422715) conducted at doses of 0, 150, 500, and 2500 ppm. Signs of toxicity were limited to the 2500 ppm group and included: (i) decreased body weight and body weight gains throughout the pre-mating, gestation, and lactation phases in the parents and from PND 4 through weaning in the F1- and F2-generations; (ii) decreased absolute thymus weights in the P-, F1- and F2-generations; and (iii) delayed sexual maturation in both sexes. The high dose of 2500 ppm was considered to exceed the maximum tolerated dose; therefore, 2000 ppm was selected to be the high dose for this study.

6. **Preparation of test articles:** Formulations (diet admixtures) containing the test substance in acetone at each of three concentrations were prepared by the Diet Preparation Lab at Bayer CropScience LP (Stilwell, KS). The formulations were shipped as needed throughout the course of the study and were stored refrigerated (2-8°C). The formulations were aliquotted for weekly use (stored at room temperature during each week of use). Further details of the dose preparation were not provided.

   CPS was used as a positive control and was stored frozen (-10 to -30°C) as a white powder. CPS was administered in phosphate buffered saline. Positive control formulations were prepared and/or aliquotted once on or before the day(s) of administration.

   Candida albicans formalin fixed cells and *Candida albicans* chitosan antigen were stored frozen (-10 to -30°C). These preparations were performed by ImmunoTox, Inc. personnel at the Testing Facility on the days of administration. Preparation procedures for the immunological materials were documented and maintained with the data generated by ImmunoTox, Inc. (data not found). Sheep red blood cells (SRBCs) were stored refrigerated (2-8°C).

7. **Dose formulation analysis:** Homogeneity (top, middle, and bottom strata) and stability (for up to 21 days in the refrigerator or at room temperature) were confirmed in a previously conducted 28-day toxicity/immunotoxicity study (Argus Study No. RLF00001) at concentrations of 150 and 300 ppm. In the present study, concentrations were measured in

the formulations at each dose level every other week, a total of 5 times during the study.

Results

Homogeneity (range as %RSD): 1.0-2.0%

Stability (% of initial concentration [range includes refrigerated and room temperatures]): 96-100%

Concentration (range as % of nominal): 90-105%

The analytical data indicated that the mixing procedure was adequate and that the variation between nominal and actual dosage to the animals was acceptable.

C. METHODS AND OBSERVATIONS

1. **Maternal animals:** Rats were observed for viability at least twice each day of the study. Rats were observed weekly for clinical signs during the pre-exposure period, on GD 0, and daily thereafter. An individual unaware of each rat's dosage group examined the rats at GD 6 and daily thereafter outside the home cage for signs of autonomic function, abnormal postures, abnormal movements, abnormal behavior patterns, and unusual appearance. Assessment of autonomic function included evaluation of lacrimation, salivation, palpebral closure, prominence of the eye, piloerection, respiration, urination, and defecation.

   Body weights were recorded weekly during pre-exposure, on GD 0, daily during all other periods, and prior to sacrifice. Feed consumption was recorded on GD 0 and daily during all other periods, but was not tabulated after LD 13 because the pups began to consume maternal feed.

   Rats were evaluated for adverse clinical signs observed during parturition, and duration of gestation, litter size, live litter sizes, and pup viability at birth were determined. Maternal behavior was evaluated on LD 0, 4, 7, 13, and 21. Maternal behavior was also evaluated on LD 12 and 18 for a few of these rats.

   Rats were sacrificed by carbon dioxide asphyxiation on GD 25 (rats not delivering) or LD 21. Necropsies were performed; the number and distribution of implantation sites were recorded. Uteri of rats that did not deliver a litter were examined while being pressed between glass plates to confirm the absence of implantation sites. Gross lesions were retained in neutral buffered 10% formalin for possible future evaluation.

   A dam with no surviving pups was sacrificed after the last pup was found dead or missing. A rat that died was examined for cause of death, necropsied, and pregnancy status and uterine contents were determined. Surviving pups of this dead rat were sacrificed.

2. **Offspring:** PND 0 was defined as the day of birth, and all pups were weighed individually at PND 0. Each litter was evaluated for viability at least twice daily, and pups were counted

once daily. Clinical observations were generally recorded once daily during the pre-weaning period. Pup body weights were recorded on PND 0, 4, 7, 11, 13, 17, and 21.

Pups that died before initial examination of the litter for pup viability were evaluated for vital status at birth. The lungs were removed and immersed in water. Pups with lungs that sank were considered stillborn. When the lungs floated, the pups were considered liveborn and to have died shortly after birth. Pups found dead were examined for gross lesions and for cause of death. Pups found dead during PND 0-4 were preserved in Bouin's solution for possible future evaluation, and pups found dead during PND 5-21 were preserved in neutral buffered 10% formalin. On PND 3 or 4, all pups not selected for continued evaluation were sacrificed by carbon dioxide asphyxiation and examined for gross lesions.

The F1 weanlings not selected for immunoassay (2-3 rats/sex/dose) were observed for viability at least twice daily and examined for clinical signs once weekly. Body weights were recorded weekly and prior to sacrifice. Food consumption values were also recorded weekly. Rats were sacrificed at approximately 7 weeks of age and were examined for gross lesions.

C. IMMUNOTOXICITY

1. **Assay 1 (anti-SRBC antibody forming cell assay):** anti-SRBC antibody forming cell assay was conducted to evaluate humoral immunity. Four days prior to their sacrifice, 0.5 ml sheep red blood cells (SRBC) were administered intravenously to F1 weanlings rats (20/sex/group) at dose levels of 0, 150, 500, or 2000 ppm clothianidin and a positive control group (10/sex) with cyclophosphamide (50mg/kg, i.p.). The day 4 response following sensitization had been previously determined as the optimum response day in rats[1]. Rats were sacrificed at 41±5 days of age and were examined for gross lesions. The thymus and spleen were excised and weighed. The thymus and any gross lesions were retained in neutral buffered 10% formalin for possible future evaluation. All spleens were harvested aseptically, weighed under sterile conditions, and processed for shipment. Spleens were stored in Earle's Balanced Salt Solution in HEPES medium with gentamicin under refrigeration (2-8°C). Spleens were shipped on wet ice or cold packs on the day of harvest via overnight courier to ImmunoTox, Inc. (Richmond, VA) for assay. Single-cell suspensions of spleen cells were made using a Stomacher® 80 Lab Blender. Primary antibody (IgM) response to SRBC was measured using a modified hemolytic plaque assay of Jerne[2]. A plaque, occurring from the lysis of SRBC, is elicited as a result of the interaction of complement and antibodies (produced in response to the i.v. sensivization) directly against SRBC. Each plaque is generated from a single antibody-producing B cell or antibody-forming - cells (AFC). AFC were counted using a Bellco plaque viewer. The data are expressed as AFC/10^6 spleen cells (specific activity), and AFC/spleen (total spleen activity).

2. **Assay 2 (delayed-type hypersensitivity):** Delayed-type hypersensitivity (DTH) assay was conducted to evaluate cell-mediated immunity by measuring the response to the challenge of

[1] Temple, L., Kawabata, T.T., et. al. (1993). Comparison of ELISA and plaque-forming cell assay for measuring the humoral immune response to sRBC in animals treated with benzo(a)pyrene or cyclophosphamide. *Fund. Appl. Toxicol.* 21:412-419.

[2] Jerne NK, Henry C, *et. al.* (1974). Plaque-forming cells: Methodology and theory. *Trnspl. Rev.* 18:130-191.

T-cell antigen, *Candida albicans*. F1 weanlings (20/sex/group and 10/sex for positive control group) were first sensitized with 0.2 ml of *Candia albicabs* formalin fixed cells (approximately 10^7 organisms), administered in the right flank subcutaneously. After eight days, the animals were challenged in the footpad with 100 µl (1.1 mg/ml) of the *Candida albicans* chitosan antigen (a purified form of the *Candia albicans* antigen). Swelling of the footpad, due to the DTH response, was measured using a micrometer at approximately 24 and 48 hours after challenge. A challenge-only control group of rats was used to correct for trauma associated with the challenge injection.

D. DATA ANALYSIS: The following statistical analyses were performed with statistically significant probabilities reported as $p \leq 0.05$ or $p \leq 0.01$.

PARAMETER	STATISTICAL ANALYSES
Clinical signs Other proportion data	The Variance Test for Homogeneity of the Binomial Distribution (Snedecor and Cochran, 1967) was conducted.
Body weights Body weight gains Food consumption Organ weights	Data were analyzed using Bartlett's Test of Homogeneity of Variances ($p=0.001$). If the variances were homogeneous, a one-way analysis of variance (ANOVA) was performed, followed by Dunnett's Test if the ANOVA was significant ($p \leq 0.05$). When the variance was heterogeneous, the Kruskal-Wallis test was performed when ≤75% ties were present, followed by Dunn's Method of Multiple Comparisons if the Kruskal-Wallis test was significant ($p \leq 0.05$). If there were >75% ties, Fisher's Exact Test was used to analyze the data.
Count data	Data were evaluated using the Kruskal-Wallis test, followed by Dunn's Method of Multiple Comparisons when appropriate.
Immunoassay data including: Body weight Thymus weight Spleen weight Foot pad swelling No. of spleen cells IgM AFC/10^6 spleen cells IgM AFC/spleen	Data were analyzed using Bartlett's Chi Square Test. If the variances were homogeneous, a one-way ANOVA was performed, followed by Dunnett's Test if the ANOVA was significant. When the variance was heterogeneous, the Kruskal-Wallis test was performed, followed by the Gehan-Wilcoxon Test if the Kruskal-Wallis test was significant. The Jonckheere's Test was used to test for dose level-related trends across the carrier and exposed groups. The positive control was compared to the carrier group using the Student's t-test.

These statistical analyses were generally considered appropriate; however, the assumption of normal data distribution should have also been tested prior to the use of parametric analysis.

II. RESULTS

A. MATERNAL TOXICITY

1. **Mortality and clinical signs of toxicity:** No treatment-related effects were observed on mortality. One 2000 ppm dam died on LD 21, but this death was considered incidental. The only adverse clinical sign noted for this rat prior to its death was a mass located on the lower midline on LD 11. This rat's body weight, food consumption, and gross pathological findings were unremarkable. The rat gave birth to a litter of 15 pups.

   An increased ($p \leq 0.01$) incidence of ptosis was observed at 2000 ppm in 6 animals for a total of 16 observations compared to 0 animals in the other groups. The incidence of other clinical signs and autonomic function findings in the treated groups were similar to controls.

2. **Body weight:** During gestation period, at 2000 ppm, body weights were generally decreased (↓3-6%). Body weight gains were decreased ($p \leq 0.01$) during GD 6-9 by 71% and during GD 18-20 by 25% (Table 2). These effects contributed to a decrease ($p \leq 0.01$) in body weight gain of 21% for the overall (GD 6-20) treatment period and a decrease ($p \leq 0.01$) in the overall (GD 0-20) gestational period body weight gain of 14%.

   During lactation period, at 2000 ppm, body weights were generally decreased (↓3-11%), a weight loss ($p \leq 0.01$) was noted during LD 0-3 (-8.0 g) compared to controls (4.6 g), and body weight gain remained decreased ($p \leq 0.01$) for LD 3-6 (↓58%). However, body weight gain for the overall (LD 0-21) lactation period was unaffected, due to increases ($p \leq 0.05$) in body weight gain for LD 9-13 and 16-21. A body weight loss ($p \leq 0.01$) was also noted during LD 0-3 at 500 ppm (-5.1 g) compared to controls (4.6 g), which contributed to a decreased ($p \leq 0.01$) overall (LD 0-21) body weight gain of 32%. As the effect on overall body weight gain during the lactation period was unrelated to dose, and no other difference ($p \leq 0.05$) in body weights or body weight gains were noted at 500 ppm throughout gestation and lactation, the isolated incidence at LD 0-3 was not considered an adverse affect on body weight gain. Body weight gain was similar to controls in the other treated groups.

TABLE 2. Mean (±SD) maternal body weight gain (g) [a]

Interval	Dose (ppm)			
	0	150	500	2000
Pre-treatment: GD 0-6	27.4±9.0	28.2±5.6	27.2±6.7	30.6±6.8
Treatment: GD 6-9	15.2±6.8	14.8±4.3	16.6±5.0	4.4±7.5** (↓71)
GD 18-20	23.2±4.2	22.7±8.2	18.8±15.6	17.5±7.6** (↓25)
GD 6-20	111.3±11.0	107.0±24.6	105.4±18.1	88.2±11.7** (↓21)
Overall: GD 0-20	138.8±15.1	135.2±24.6	132.6±19.9	119.0±11.5** (↓14)
LD 0-3	4.6±9.5	1.4±9.1	-5.1±11.0** (↓211)	-8.0±11.5** (↓274)
LD 3-6	11.8±6.9	13.7±4.6	9.2±7.6	5.0±8.8** (↓58)
LD 9-13	4.4±14.3	2.0±14.3	1.6±11.1	12.4±10.5* (↑182)
LD 16-21	1.4±14.5	2.7±14.5	0.0±9.0	12.9±6.2* (↑821)
Overall: LD 0-21	43.4±12.3	36.5±12.3	29.6±14.8** (↓32)	43.7±17.0 (↑1)

a Data (n=19-25) were obtained from Table A5 on page 63 and Table A7 on page 65 of the study report. Percent differences from the control group, calculated by the reviewers, are included in parentheses.

** Statistically different ($p \leq 0.01$) from the control.

3. **Food consumption:** At 2000 ppm, food consumption (g/rat/day and g/kg bw/day) was decreased ($p \leq 0.01$) for the overall treatment (↓14-17%), gestational (↓8-11%), and lactation periods (↓17-23%; Tables 3a and 3b). Food consumption (g/rat/day and g/kg bw/day) was also slightly decreased ($p \leq 0.01$) at 500 ppm throughout lactation (↓11-18%) and for the overall lactation period (↓12-13%). Differences ($p \leq 0.05$) were sporadically observed at 150 ppm, but were considered incidental.

TABLE 3a. Mean (±SD) maternal absolute food consumption (g/day) [a]				
Interval	Dose (ppm)			
	0	150	500	2000
Pre-treatment: GD 0-6	18.0±1.5	18.3±1.4	18.8±1.6	19.0±1.6
Treatment: GD 6-20	20.6±1.4	20.3±1.4	20.4±1.5	17.2±1.6** (↓17)
Overall: GD 0-20	19.8±1.3	19.7±1.2	19.9±1.3	17.7±1.4** (↓11)
Overall: LD 0-21	54.4±3.9	53.5±3.5	47.3±8.5** (↓13)	41.8±4.8** (↓23)

a Data (n=21-25) were obtained from Table A8 on page 66 and Table A10 on page 68 of the study report. Percent differences from the control group, calculated by the reviewers, are included in parentheses.
** Statistically different (p≤0.01) from the control.

TABLE 3b. Mean (±SD) maternal relative food consumption (g/kg/day) [a]				
Interval	Dose (ppm)			
	0	150	500	2000
Pre-treatment: GD 0-6	77.1±5.3	78.7±4.6	80.5±4.9	80.6±5.8
Treatment: GD 6-20	69.9±4.1	69.6±3.0	70.1±3.7	60.3±4.8** (↓14)
Overall: GD 0-20	68.3±3.4	68.7±2.4	69.4±2.9	63.0±4.2** (↓8)
Overall: LD 0-21	184.5±10.6	179.0±10.6	162.9±27.2** (↓12)	153.2±11.8** (↓17)

a Data (n=24-25) were obtained from Table A9 on page 67 and Table A11 on page 69 of the study report. Percent differences from the control group, calculated by the reviewers, are included in parentheses.
** Statistically different (p≤0.01) from the control.

4. **Gross pathology**: No treatment-related finding was observed during gross pathology.

5. **Reproductive performance**: No treatment-related effect was noted on the reproductive performance parameters (Table 4). There was a decreased (p≤0.01) number of females pregnant at 150 ppm, but this finding was unrelated to dose.

TABLE 4. Reproductive performance [a]				
Observation	Dose Group (ppm)			
	0	150	500	2000
Number in group (both sexes)	25	25	25	25
Intercurrent deaths (females)	0	0	0	0
Mean (±SD) gestation interval (days)	22.4±0.5	22.5±0.6	22.4±0.6	22.6±0.6
Number pregnant	24	21**	25	25
Number of litters	24	20	25	25
Gestation index [b]	100	95.2	100	100

a Data were obtained from Table A12 on page 70 of the study report.
b Number of rats with live offspring/number of pregnant rats x 100
** Statistically different (p≤0.01) from the control.

B. OFFSPRING

1. **Viability and clinical signs**: Total implantation sites, litter size, and litter viability are presented in Table 5. No treatment-related effects were observed on these parameters. A decreased (p≤0.05) number of surviving pups/litter was observed at 500 ppm on Day 0 (11.8 treated vs 13.9 controls), but this finding was unrelated to dose. Furthermore, no mortality or clinical signs of toxicity were observed in the treated groups after weaning.

TABLE 5. Mean ±SD Litter parameters [a]

Observation	Dose Group (ppm)			
	0	150	500	2000
Total number implantation sites in P	349	260	325	341
(Implantations/dam)	14.5±1.2	13.0±3.2	13.0±2.8	13.6±1.8
Total number of liveborn pups	334	245	294	326
Liveborn pups/dam	13.9±1.5	12.2±3.4	11.8±3.7*	13.0±1.7
Total number of stillborn pups	0	0	1	3
Total no. of pups born of unknown vital status	1	0	1	0
Mean % males per litter	49.4±13.4	52.8±19.5	51.3±19.2	51.4±14.8
Deaths on Days 0-4 [b]	4	2	3	4
Deaths on Days 4-21 [b]	0	2	0	0
Surviving pups/litter on Day 0	13.9±1.5	12.2±3.4	11.8±3.7*	13.0±1.7
Day 4 [c]	13.7±1.4	11.8±4.1	11.0±4.3	12.9±1.8
Day 4 [d]	10.0±0.0	9.4±2.2	9.2±2.4	9.9±04
Day 7	10.0±0.0	9.4±2.2	9.2±2.4	9.9±0.4
Day 13	10.0±0.0	9.4±2.2	9.2±2.4	9.9±0.4
Day 21	10.0±0.0	9.4±2.2	9.2±2.4	9.9±0.4
Gestation index [e]	100	95.2	100	100
Viability index [f]	98.8	99.1	98.9	98.8
Lactation index [g]	100	98.9	100	100

a Data were obtained from Tables A12 and A13 on pages 70-73 in the study report.
b Calculated by the reviewer from data presented in Table A13 on page 71.
c Before standardization (culling)
d After standardization (culling)
e Number of rats with live offspring/number of pregnant rats x 100
f Number of live pups on PND 4 (preculling)/number of liveborn pups on PND 0 x 100
g Number of live pups on PND 21 (weaning)/number of live pups on PND 4 (postculling) x 100
* Statistically different ($p \leq 0.05$) from the control.

2. Body weight

a. Pups prior to weaning: Mean pup weights in the 2000 ppm treated group were decreased ($p \leq 0.01$) throughout lactation (↓12-26%; Table 6). Pup weights in the 150 and 500 ppm groups were similar to controls throughout lactation, except for an incidental increase ($p \leq 0.05$) at 150 ppm of 7% on PND 4 (post-culling).

TABLE 6a. Mean (±SD) pup weights (g) [a]

LD	Dose Group (ppm)			
	0	150	500	2000
0	6.4±0.4	6.5±0.5	6.5±0.5	6.4±0.5
4 [b]	10.3±0.9	10.8±1.3	10.6±1.0	9.1±1.1** (↓12)
4 [c]	10.4±0.9	11.1±1.2* (↑7)	10.7±1.0	9.2±1.0** (↓12)
7	16.1±1.7	16.5±1.1	15.8±2.0	12.7±1.8** (↓21)
21	45.4±5.4	46.4±3.8	42.8±5.4	33.8±5.6** (↓26)

a Data (n=17-25) were obtained from Table A13 on page 75 of the study report.
b Before standardization (culling)
c After standardization (culling)
* Statistically different ($p \leq 0.05$) from the control.
** Statistically different ($p \leq 0.01$) from the control.

b. F1 offspring post-weaning: In Assays 1 and 2 tested animals, changes ($p \leq 0.05$) in body weight and body weight gains at 150 and 500 ppm post-weaning were generally slight and were not considered adverse. At 2000 ppm post-weaning, decreased ($p \leq 0.01$) body weight and body weight gains were generally observed until termination (↓19-29%), and bodyweight gain from Day 22 until termination was decreased ($p \leq 0.01$) by 24-25% in males and 15-17% in females (Tables 6b and 6c).

TABLE 6b. Mean (±SD) F1-generation rat weights (g) in Assay 1 [a]

Day	Dose Group (ppm)			
	0	150	500	2000
Males				
22	50.4±7.1	52.4±5.0	46.2±6.0** (↓8)	36.2±7.1** (↓28)
29	91.7±8.9	97.0±7.1	84.8±8.6* (↓8)	67.6±10.7** (↓26)
36	147.3±12.0	152.0±13.1	138.4±12.0* (↓6)	109.5±14.3** (↓26)
Terminal	202.0±14.8	214.0±11.7* (↑6)	198.6±15.2	150.2±19.3** (↓26)
BWG (Days 22-29)	43.5±4.7	44.6±3.5	39.1±4.3** (↓10)	31.4±4.6** (↓28)
BWG (Days 29-36)	55.6±5.4	55.0±7.8	53.6±6.0	41.8±4.7** (↓25)
BWG (Days 22-Termination)	152.8±12.9	162.0±11.4* (↑6)	152.4±12.4	114.2±15.7** (↓25)
Females				
22	48.0±6.5	49.8±4.4	45.9±6.5	34.5±7.2** (↓28)
29	85.2±6.9	87.8±4.8	81.2±8.6	60.2±13.5** (↓29)
36	125.0±7.4	128.4±6.2	121.2±9.5	94.0±16.6** (↓25)
Terminal	161.2±9.8	164.2±11.2	159.9±13.4	128.0±14.9** (↓21)
BWG (Days 22-29)	37.7±3.4	38.3±3.2	35.3±4.7* (↓6)	28.3±5.9** (↓25)
BWG (Days 29-36)	39.8±3.6	40.5±2.7	40.0±4.8	33.8±4.3** (↓15)
BWG (Days 22-Termination)	113.4±9.1	114.8±10.0	114.0±13.4	94.0±10.5** (↓17)

a Data were obtained from Tables B3-B4 on pages 188-189 and Tables C3-C4 on pages 249-250 of the study report.
* Statistically different ($p \leq 0.05$) from the control.
** Statistically different ($p \leq 0.01$) from the control.

TABLE 6c. Mean (±SD) F1-generation rat weights (g) in Assay 2 [a]				
Day	Dose Group (ppm)			
	0	150	500	2000
Males				
22	50.4±7.1	52.4±5.0	46.2±6.0** (↓8)	36.2±7.1** (↓28)
29	96.0±9.6	96.8±6.5	85.8±7.6** (↓11)	67.8±10.5** (↓29)
36	151.0±13.4	153.4±9.4	138.8±11.0** (↓8)	111.1±14.0** (↓26)
Terminal	205.0±21.0	217.0±13.5	197.3±18.4	152.8±20.0** (↓25)
BWG (Days 22-29)	43.5±4.7	44.6±3.5	39.1±4.3** (↓10)	31.4±4.6** (↓28)
BWG (Days 29-36)	55.0±6.1	56.6±5.1	53.0±5.3	43.4±5.3** (↓21)
BWG (Days 22-Termination)	153.4±18.6	164.3±10.4* (↑7)	151.2±15.5	116.2±13.4** (↓24)
Females				
22	48.0±6.5	49.8±4.4	45.9±6.5	34.5±7.2** (↓28)
29	86.2±6.9	88.2±5.4	81.2±7.8* (↓6)	65.6±8.5** (↓24)
36	127.2±8.4	129.2±6.8	122.5±10.1	101.0±10.8** (↓21)
Terminal	157.6±12.3	163.2±13.2	155.5±13.6	128.4±14.7** (↓19)
BWG (Days 22-29)	37.7±3.4	38.3±3.2	35.3±4.7* (↓6)	28.3±5.9** (↓25)
BWG (Days 29-36)	41.0±5.8	40.9±3.0	41.4±3.8	35.4±4.0** (↓14)
BWG (Days 22-Termination)	109.4±13.4	113.1±12.4	109.6±11.4	93.4±10.5** (↓15)

a Data were obtained from Tables B3-B4 on pages 188-189 and Tables C3-C4 on pages 249-250 of the study report.
* Statistically different ($p \leq 0.05$) from the control.
** Statistically different ($p \leq 0.01$) from the control.

3. **Food consumption**: In the F1 rats, decreased ($p \leq 0.05$) absolute food consumption was noted during the interval of PND 22-29 at 500 ppm in males (↓13%) and females (↓7%; Tables 7a and 7b). Absolute food consumption (g/rat/day) was decreased ($p \leq 0.01$) at 2000 ppm for PND 22-29, 29-36, and 22-36 (↓19-34%). Additionally, decreased ($p \leq 0.05$) food consumption was observed in the 500 ppm females in Assay 2 for PND 29-36 and 22-36 (↓7%). However, relative food consumption (g/kg/day) only showed minor effects.

TABLE 7a. Mean (±SD) F1-generation absolute food consumption (g/rat/day) in Assay 1 [a]				
Day	Dose Group (ppm)			
	0	150	500	2000
Males				
22-29	12.6±2.3	12.6±2.0	10.9±2.6** (↓13)	8.5±1.5** (↓33)
29-36	18.7±2.2	18.4±1.5	17.5±1.6	14.4±2.2** (↓23)
22-36	18.6±2.1	18.3±1.4	17.5±1.6	14.3±2.1** (↓23)
Females				
22-29	11.9±1.0	11.9±0.9	11.1±2.2* (↓7)	8.5±1.9** (↓29)
29-36	16.0±1.5	15.6±1.2	15.3±1.4	12.6±1.8** (↓21)
22-36	16.0±1.4	15.6±1.2	15.3±1.4	12.5±1.8** (↓22)

a Data were obtained from Tables B5 (pages 190) and C5 (page 251) of the study report.
* Statistically different ($p \leq 0.05$) from the control.
** Statistically different ($p \leq 0.01$) from the control.

TABLE 7b. Mean (±SD) F1-generation absolute food consumption (g/rat/day) in Assay 2 [a]				
Day	Dose Group (ppm)			
	0	150	500	2000
Males				
22-29	12.6±2.3	12.6±2.0	10.9±2.6** (↓13)	8.5±1.5** (↓33)
29-36	18.3±2.8	19.0±1.3	15.8±3.9	12.1±4.4** (↓34)
22-36	18.2±2.8	18.9±1.3	15.9±3.8	12.0±4.4** (↓34)
Females				
22-29	11.9±1.0	11.9±0.9	11.1±2.2* (↓7)	8.5±1.9** (↓29)
29-36	16.5±1.5	15.9±1.2	15.3±2.2* (↓7)	13.3±1.0** (↓19)
22-36	16.5±1.4	15.9±1.2	15.3±1.9* (↓7)	13.3±1.1** (↓19)

a Data were obtained from Tables B5 (pages 190) and C5 (page 251) of the study report.
* Statistically different ($p \leq 0.05$) from the control.
** Statistically different ($p \leq 0.01$) from the control.

4. Offspring postmortem results

a) Organ weights: No adverse, treatment-related effects were observed on thymus and spleen weights. Decreased ($p \leq 0.01$) terminal body weights and absolute spleen and thymus weights were noted in the 2000 ppm group (↓21-26%; Table 8). No effects were observed on relative (to body) organ weights. The effects on absolute organ weights were considered related to the decreased terminal body weights rather than test compound toxicity.

TABLE 8. Selected mean absolute and relative (to body) organ weights (±SD) in F1-generation rats treated with Clothianidin in Assay 1 [a]

Organ		Dose (ppm)			
		0	150	500	2000
Males					
Terminal body weight (g)		202.0±14.8	214.0±11.7* (↑6)	198.6±15.2	150.2±19.3** (↓26)
Spleen	absolute (g)	0.71±0.11	0.73±0.14	0.70±0.12	0.54±0.08** (↓24)
	relative (%)	0.355±0.055	0.341±0.054	0.351±0.052	0.365±0.050
Thymus	absolute (g)	0.72±0.15	0.77±0.12	0.77±0.14	0.57±0.08** (↓21)
	relative (%)	0.356±0.073	0.358±0.046	0.386±0.064	0.386±0.065
Females					
Terminal body weight (g)		161.2±9.8	164.2±11.2	159.9±13.4	128.0±14.9** (↓21)
Spleen	absolute (g)	0.49±0.09	0.45±0.07	0.47±0.10	0.38±0.08** (↓22)
	relative (%)	0.304±0.056	0.275±0.040	0.291±0.046	0.305±0.086
Thymus	absolute (g)	0.57±0.09	0.60±0.06	0.60±0.08	0.45±0.08** (↓21)
	relative (%)	0.356±0.068	0.367±0.047	0.378±0.038	0.355±0.053

a Data were obtained from Tables B8 (page 193) and C8 (page 254) of the study report. Numbers listed parenthetically represent the percent difference from controls (calculated by reviewers).
* Statistically different ($p \leq 0.05$) from the controls
** Statistically different ($p \leq 0.01$) from the controls

b) Macroscopic examination: No treatment-related effects were observed during necropsy.

5. Assay 1 (antibody-forming cell response): For males, significant decreases ($p \leq 0.01$, except as noted) were noted in body weights (↓26%), spleen weight (↓24%) and total spleen cell numbers (↓24%) at 2000 ppm (Table 9a). The decreases in spleen weight and spleen cells were expected due to decreased terminal body weights. Decreased spleen specific activity (AFC/10^6 spleen cells) and total spleen activity (AFC/spleen) were noted at 2000 ppm while increased activities were observed at 150 and 500 ppm; however, no statistical significances were observed in the treated groups compared with controls. The positive control induced the expected response that produced statistically significant decreases in cell number (↓83%), specific activity (↓88%) and total spleen activity (↓98%) compared to the control animals.

TABLE 9a. [a]

Spleen Primary IgM Antibody-Forming Cell Response to T-dependent Antigen Sheep Erythrocytes In F1 Generation Male Crl:CD(SD) Rats Exposed to Clothianidin During Maternal Gestation (*In Utero* Exposure), Via Maternal Milk During the Lactation Period and Orally - Day 4 Response

5819-008

Exposure	Body Wgt (g)	Spleen Wgt (mg)	Spleen Cells ($x10^7$)	IgM AFC/ 10^6 Spleen Cells	IgM AFC/Spleen ($x\ 10^3$)
Carrier	202.0 ± 3.3 (20)	714 ± 24 (20)	77.91 ± 3.42 (20)	129 ± 26 (20)	111 ± 27 (20)
Clothianidin					
150 ppm	223.1 ± 8.5** (20)	753 ± 29 (20)	85.79 ± 5.27 (20)	278 ± 49** (20)	228 ± 40** (20)
500 ppm	198.7 ± 3.4 (20)	699 ± 27 (20)	84.97 ± 4.50 (20)	200 ± 35 (20)	172 ± 33 (20)
2000 ppm	150.2 ± 4.3** (20)	546 ± 19** (20)	59.53 ± 2.74** (20)	73 ± 16 (20)	43 ± 9 (20)
Cyclophosphamide					
50 mg/kg	178.1 ± 4.2** (10)	258 ± 18** (10)	12.98 ± 1.24** (10)	16 ± 7** (10)	2 ± 1** (10)
H/NH	NH	H	NH	NH	NH
Trend Analysis	p ≤ 0.01	p ≤ 0.01	p ≤ 0.01	p ≤ 0.05	p ≤ 0.01

a Scanned copy of Table 3 on page 526 of the study report. The numbers of rats evaluated are included in parentheses.

For females, significant decreases ($p \leq 0.01$) were noted in body weight (↓21%), spleen weight (↓22%), and spleen cells (↓22%) at 2000 ppm (Table 9b). The decreases in spleen weight and spleen cells were due to decreased terminal body weights. There were statistically significant increases ($p \leq 0.05$) in spleen specific activity (AFC/10^6 spleen cells, ↑143%) and total spleen activity (AFC/Spleen, ↑99%). However, individual animal data showed that the majority of animals in this group showed similar response compared with other treated and control groups except 3 females in this group had higher specific activity and total spleen activity than the median response for this group, and resulted in the statistically significant increased activities of this group compared to the controls. The variability of immune response was commonly observed in the outbred SD rats. Therefore, the statistical significance may not be biologically significant. The positive control induced the expected response that produced significant decreases in total spleen cell number (↓84%), specific activity (↓92%) and total spleen activity (↓99%) compared with the control.

TABLE 9b. [a]

Spleen Primary IgM Antibody-Forming Cell Response to T-dependent Antigen Sheep Erythrocytes in F1 Generation Female Crl:CD(SD) Rats Exposed to Clothianidin During Maternal Gestation (*In Utero* Exposure), Via Maternal Milk During the Lactation Period and Orally - Day 4 Response

5819-008

Exposure	Body Wgt (g)	Spleen Wgt (mg)	Spleen Cells ($x10^7$)	IgM AFC/ 10^6 Spleen Cells	IgM AFC/Spleen ($x\ 10^3$)
Vehicle	161.2 ± 2.2 (20)	490 ± 20 (20)	51.35 ± 2.23 (20)	286 ± 82 (20)	140 ± 39 (20)
Clothianidin					
150 ppm	164.3 ± 2.5 (20)	453 ± 15 (20)	47.42 ± 4.32 (20)	218 ± 46 (20)	95 ± 22 (20)
500 ppm	159.9 ± 3.0 (20)	469 ± 21 (20)	48.75 ± 2.97 (20)	211 ± 46 (20)	100 ± 17 (20)
2000 ppm	128.1 ± 3.3** (20)	383 ± 19** (20)	40.14 ± 1.91** (20)	596 ± 148** (20)	278 ± 57* (20)
Cyclophosphamide					
50 mg/kg	140.6 ± 2.0** (10)	195 ± 8** (10)	8.24 ± 0.86** (10)	24 ± 9* (10)	2 ± 1* (10)
H/NH	H	H	NH	NH	NH
Trend Analysis	p ≤ 0.01	p ≤ 0.01	p ≤ 0.01	p ≤ 0.01	p ≤ 0.01

a Scanned copy of Table 4 on page 527 of the study report. The numbers of rats evaluated are included in parentheses.

6. **Assay 2 (delayed-type hypersensitivity):** As shown in Tables 10a and 10b, no treatment-related effect was observed at any treated dose at both 24 and 48 hours in this assay for males and females. The positive control showed significantly suppressive responses at 24 hour and 48 hour post-challenge.

TABLE 10a. [a]

Delayed-Type Hypersensitivity Response in F1 Generation Male Crl:CD(SD) Rats Exposed to Clothianidin During Maternal Gestation (*In Utero* Exposure), Via Maternal Milk During the Lactation Period and Orally - Day 4 Response

5819-008

Exposure	24-HR Post Measure (mm x 100)	48-HR Post Measure (mm x 100)
Challenge Only	12 ± 8** (10)	22 ± 9 (10)
Carrier	56 ± 9 (20)	40 ± 8 (20)
Clothianidin		
150 ppm	65 ± 10 (20)	49 ± 7 (20)
500 ppm	75 ± 9 (20)	61 ± 8 (20)
2000 ppm	56 ± 7 (20)	51 ± 5 (20)
Cyclophosphamide		
50 mg/kg	16 ± 11* (9)	13 ± 10 (9)
H/NH	H	H
Trend Analysis	NS	NS

a Scanned copy of Table 5 on page 528 of the study report. The numbers of rats evaluated are included in parentheses.

TABLE 10b.[a]

Delayed-Type Hypersensitivity Response in F1 Generation Female Crl:CD(SD) Rats Exposed to Clothianidin During Maternal Gestation (*In Utero* Exposure), Via Maternal Milk During the Lactation Period and Orally - Day 4 Response

5819-008

Exposure	24-HR Post Measure (mm x 100)	48-HR Post Measure (mm x 100)
Challenge Only	4 ± 5* (10)	8 ± 6 (10)
Carrier	28 ± 8 (20)	27 ± 6 (20)
Clothianidin		
150 ppm	39 ± 10 (20)	24 ± 6 (20)
500 ppm	42 ± 9 (20)	32 ± 6 (20)
2000 ppm	29 ± 6 (20)	32 ± 5 (20)
Cyclophosphamide		
50 mg/kg	2 ± 5* (10)	14 ± 4 (10)
H/NH	H	H
Trend Analysis	NS	NS

a Scanned copy of Table 5 on page 528 of the study report. The numbers of rats evaluated are included in parentheses.

III. DISCUSSION AND CONCLUSIONS

A. **INVESTIGATORS' CONCLUSIONS:** For maternal toxicity, the LOAEL was 2000 ppm, based on reductions in body weights, body weight gains, and food consumption, as well as clinical observations of ptosis. For offspring, the LOAEL was 500 ppm based on reductions in body weights in the males. Additionally at 2000 ppm, reductions in body weights, body weight gains, and food consumption were noted in both sexes of the offspring. There were no relevant adverse effects on humoral immunity or cell-mediated immunity in either sex.

B. REVIEWER COMMENTS

1. **Maternal toxicity:** No treatment-related effects were observed on mortality or gross pathology. At 2000 ppm, an increased ($p \leq 0.01$) incidence of ptosis was observed in 6 animals for a total of 16 observations compared to 0 animals in the other groups. The body weights were generally decreased ($p \leq 0.05$) during GD 9-20 (↓3-6%) and LD 0-21 (↓3-11%). Body

weight gains were decreased ($p \leq 0.01$) during GD 6-9 by 71% and during GD 18-20 by 25%. These effects contributed to a decreased ($p \leq 0.01$) body weight gain of 21% for the overall (GD 6-20) treatment period and a decreased ($p \leq 0.01$) body weight gain in the overall (GD 0-20) gestation period of 14%. During the lactation period, a weight loss ($p \leq 0.01$) was noted during LD 0-3 (-8.0 g) compared to controls (4.6 g), and body weight gain remained decreased ($p \leq 0.01$) for LD 3-6 (↓58%). Food consumption (g/rat/day and g/kg bw/day) was decreased ($p \leq 0.01$) during treatment for the overall treatment (↓14-17%), gestation (↓8-11%), and lactation periods (↓17-23%).

Food consumption (g/rat/day and g/kg bw/day) was also slightly decreased ($p \leq 0.01$) at 500 ppm during the overall lactation period (↓12-13%); however, a dose-dependent effect was not observed on body weight gain during this period. Therefore, this slight decrease was not considered adverse.

No treatment-related effect was noted on the reproductive performance parameters.

The maternal LOAEL is 2000 ppm based on increased incidence of ptosis, and decreased body weights and body weight gain, and food consumption. The maternal NOAEL is 500 ppm.

2. **Offspring toxicity:** No treatment-related effects were observed on litter size and litter viability. No mortality or clinical signs of toxicity were observed in the treated groups after weaning. No treatment-related effects were observed on organ weights or gross pathology.

   At 2000 ppm, mean pup weights in the treated groups were decreased ($p \leq 0.01$) throughout lactation (↓12-26%). After weaning, decreased ($p \leq 0.01$) body weight and body weight gains were generally observed until termination (↓7-29%), and bodyweight gain from Day 22 until termination was decreased ($p \leq 0.01$) by 24-25% in males and 15-17% in females. Absolute food consumption was decreased ($p \leq 0.01$) for Days 22-29, 29-36, and 22-36 (↓19-34%).

   Pup weights in the 150 and 500 ppm groups were similar to controls throughout lactation, except for an incidental increase ($p \leq 0.05$) at 150 ppm of 7% on PND 4 (post-culling). In the F1 weanlings, changes ($p \leq 0.05$) in body weight and body weight gains at 150 and 500 ppm were generally slight and were not considered adverse. Decreased ($p \leq 0.05$) absolute food consumption was noted during the interval of Days 22-29 at 500 ppm in males (↓13%) and females (↓7%). Additionally, decreased ($p \leq 0.05$) food consumption was observed in the 500 ppm females in Assay 2 for Days 29-36 and 22-36 (↓7%). These effects were considered slight and did not affect body weight appreciably.

   The offspring LOAEL is 2000 ppm based on a decreased body weights, body weight gains, and food consumption. The offspring NOAEL is 500 ppm.

3. **Immunotoxicity:** There were statistically significant decreases in thymus and spleen weight and spleen cell number in the F1 weanlings at 2000 ppm for males and females. These decreases might be related to significant decrease of body weight at the high dose and was

considered secondary effect to general systemic toxicity and not direct immunological toxicity.

For humoral immunity, the antibody-forming cell assay in males showed decreases in spleen specific activity (AFC/10^6 spleen cells) and total spleen activity (AFC/spleen) at 2000 ppm while increased activities were observed at 150 and 500 ppm; however, no statistical significances were observed in the treated groups compared with controls. In addition, females showed statistically significant increases ($p \leq 0.05$) in spleen specific activity (AFC/10^6 spleen cells ↑143%) and total spleen activity (AFC/Spleen ↑99%) at 2000 ppm. However, individual animal data showed that the majority of animals in this group showed similar response compared with other treated and control groups. There were 3 females in the 2000 ppm that had higher specific activity and total spleen activity than the median response for this group that resulted in the statistically significant increased activities of this group compared to the controls. The variability of immune response was commonly observed in the outbred SD rats. Therefore, the statistical significance may not be biologically significant considering with distribution pattern of the responses. The positive control induced the expected response that produced significant decreases in total spleen cell number, specific activity and total spleen activity compared with the control.

For cell-mediated immunity, delayed-type hypersensitivity (DTH) assay did not show treatment-related effect at any treated dose at both 24 and 48 hours in this assay for males and females. The positive control showed significantly suppressive responses at 24 hour and 48 hour post-challenge.

Under conditions of this study, there were no immunologically adverse effects on humoral or cell-mediated immunity in male and female rats that exposed to clothianidin during the prenatal, postnatal and post-weaning period.

This study is classified acceptable/non-guideline and provides information for use in evaluating the potential for immunotoxic effect in offspring rats after exposure to clothianidin during the prenatal, postnatal and post-weaning period.

13544

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